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Do native and invasive herbivores have an effect on *Brassica rapa* pollination?

Scopece, G ; Frachon, Léa ; Cozzolino, Salvatore

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DOI: <https://doi.org/10.1111/plb.12985>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-170465>

Journal Article

Accepted Version

Originally published at:

Scopece, G; Frachon, Léa; Cozzolino, Salvatore (2019). Do native and invasive herbivores have an effect on *Brassica rapa* pollination? *Plant Biology*, 21(5):927-934.

DOI: <https://doi.org/10.1111/plb.12985>

1 **Do native and invasive herbivores have an effect on *Brassica rapa* pollination?**

2

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12 Running title: Herbivory and pollination in *Brassica rapa*

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15

16 Keywords: Herbivory; Honey bees; *Mamestra brassicae*; *Pieris brassicae*; *Spodoptera littoralis*;
17 wild pollinators.

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Abstract

- Mutualistic (e.g. pollination) and antagonistic (e.g. herbivory) plant-insect interactions shape levels of plant fitness and can have interactive effects.
- By using experimental plots of *Brassica rapa* plants infested with generalist (*Mamestra brassicae*) and specialized (*Pieris brassicae*) native herbivores and with a generalist invasive (*Spodoptera littoralis*) herbivore, we estimated both pollen movement among treatments and the visiting behaviour of honey bees *versus* other wild pollinators.
- Overall, we found that herbivory has weak effects on plant pollen export either in terms of inter-treatment movements or of dispersion distance. Plants infested with the native specialized herbivore tend to export less pollen on other plants with the same treatment. Other wild pollinators preferentially visit non-infested plants differently from honey bees that showed no preferences. Honey bees and other wild pollinators also showed different behaviours facing plants infested by different herbivores with the former tending to avoid revisiting the same treatment and the latter showing no avoidance behaviour. When taking into account the whole pollinator community, i.e. the interactive effects of honey bees and other wild pollinators, we found an increased avoidance of plants infested by the native specialized herbivore and a decreased avoidance of plants infested by the invasive herbivore.
- Taken together, our results suggest that herbivory may have an effect on *B. rapa* pollination but this effect depends on the relative abundance of honey bees and other wild pollinators.

41 INTRODUCTION

42

43 Plant-insect interactions are among the main factors that shape plant fitness and, consequently,
44 crop yields (e.g. Schemske & Bradshaw 1999; Cornell and Hawkins 2003; Garibaldi *et al.* 2011;
45 Rosas-Guerrero *et al.* 2014; Gómez *et al.* 2015; Stein *et al.* 2017). These interactions can be either
46 mutualistic (e.g. pollination) or antagonistic (e.g. herbivory) with expected opposite effects on
47 overall plant fitness (Herrera *et al.* 2002). Whilst the role of pollinator communities on plant fitness
48 is straightforward to assess due to their direct impact on the amount and type of pollen transfer,
49 herbivores can have direct or indirect detrimental effects on plant fitness. Herbivores can affect the
50 number of flowers produced (Litto *et al.* 2015) and decrease the plant resources allocable in seed
51 production directly influencing plant fitness (Strauss *et al.* 1996; Mothershead & Marquis 2000;
52 Knauer & Schiestl 2017) or can change the attractiveness of plants to pollinators indirectly
53 influencing plant fitness.

54 Plants communicate with insects by producing compounds that can be either attractive or repellent
55 depending on the antagonistic-mutualistic nature of the interaction (Schiestl & Johnson 2013).

56 From an evolutionary perspective, natural selection is expected to keep the communication
57 channels of these interactions separate because plants should produce both compounds attractive
58 to pollinators (but not for herbivores) and repellent for herbivores (but not for pollinators)
59 (Parachnowitsch *et al.* 2012; Knauer & Schiestl 2017). Nonetheless, in the face of herbivore
60 attacks, plants may respond by changing floral volatile production, floral display or nectar
61 production, which is likely to affect pollinator attractiveness (Herrera *et al.* 2002; Knauer & Schiestl
62 2017; Glaum & Kessler 2017). This effect can be positive or detrimental for plant fitness, i.e. it can
63 determine a higher or a lower attractiveness to pollinators (e.g. Cozzolino *et al.* 2015; Litto *et al.*
64 2015; Knauer & Schiestl 2017). Although several studies investigated the interactive effect of
65 pollinators and herbivores on plant fitness (Galen & Cuba 2001; Gómez 2003, 2005, 2008;
66 Cariveau *et al.* 2004; Rey *et al.* 2006; Parachnowitsch & Caruso 2008; Wise & Hebert 2010;
67 Sletvold *et al.* 2015; Sauve *et al.* 2016), herbivory impact on pollinator foraging preferences and
68 inter-individual gene flow within plant patches are little investigated.

69 Under herbivore attack, the subsequently induced volatiles can affect plant pollination both at
70 individual and population level (Liao *et al.* 2013). Plant populations subject to herbivore attack may
71 not only experience a reduction in individual pollination success, but also an alteration in the
72 pattern of pollinator-mediated pollen transfer within and among plant patches. In the presence of
73 induced volatiles, pollinators can change their foraging preference affecting both the pollination
74 success of healthy plants and pollen flow between attacked and healthy plants (Krupnick *et al.*
75 1999). Despite most entomophilous plant species employing highly generalist pollination strategies
76 (Johnson & Steiner 2000), most experimental studies on the effects of herbivore attacks on plant
77 responses to pollinators focused on a single insect species (e.g. Knauer & Schiestl 2017). Yet, it is
78 important to understand the joint effect of the entire pollinator community and the contribution of
79 each pollinator group (but see Rusman *et al.* 2018). For instance, by displaying different
80 behaviours, the relative abundance of honey bees and wild pollinators in a population can alter
81 local plant performances (Mallinger & Gratton 2015). Due to strong concerns that have emerged
82 over the last few decades regarding the decline in pollinators and the increase in alien herbivores,
83 understanding the interaction between pollinator and herbivore communities has become
84 particularly important (Potts *et al.* 2010; Ward & Masters 2007; Hallmann *et al.* 2017). The decline
85 of wild pollinators and honey bees have been raised worldwide (Potts *et al.* 2010; Rader *et al.*
86 2016), potentially threatening yields of economically important crops (Klein *et al.* 2007; Aizen *et al.*
87 2008; Winfree *et al.* 2008). The role and the behaviour of honey bees *versus* other wild pollinator
88 services have been highlighted, particularly due to the involvement of wild pollinators in 'pollination
89 insurance' for wild and crop species (Winfree *et al.* 2007; Rader *et al.* 2016). Coupled with this
90 pollinator decline, a worldwide spread of alien herbivores species is observed due to an increase in
91 exchanges among geographically distant areas (Ward & Masters 2007). Whilst plant species are
92 generally well adapted to local herbivores, the appearance of alien herbivores can have important
93 consequences on plant fitness. Indeed, evolutionary naiveté may cause plants to be less defended
94 against invasive species or show maladaptive responses to damage, which may affect chemical
95 signalling and interactions with pollinators (Parker *et al.* 2006; Gutbrodt *et al.* 2012; Desurmont *et*
96 *al.* 2014).

97 *Brassica rapa* is a generalist plant species that was reported to attract a wide *spectrum* of
98 pollinator insects (Atmowidi *et al.* 2007; Rader *et al.* 2009). This species is typically infested by
99 specialized insects, including caterpillars of the genus *Pieris* (Pieridae, Lepidoptera), but has also
100 been reported to be edible for some generalist herbivore species as *Mamestra brassicae*
101 (Noctuidae, Lepidoptera) and alien-invasive species as the generalist *Spodoptera littoralis*
102 (Noctuidae, Lepidoptera) (Amin & Gergis 2006).

103 In this study, by using experimental approaches with plants infested by generalist and specialised
104 local herbivores and by a generalist invasive herbivore, we estimated the cross effect of native and
105 alien herbivores on the foraging behaviour of pollinators in *B. rapa* plants and their potential impact
106 on plant pollen export. We specifically addressed the following questions:

- 107 i) Is pollen movement within *B. rapa* patches affected by the presence of native (either
108 generalist or specialized) and alien herbivores?
- 109 ii) Does the local pollinator community behave differently towards plants infested by native
110 or alien herbivores? In particular, do honey bees and other wild pollinators differ in their
111 behaviour and visiting preferences?

112

113 MATERIAL AND METHODS

114

115 Study organisms

116 *Brassica rapa* is a non-autogamous self-incompatible, annual or biennial plant species native to
117 Eurasia (Watanabe *et al.* 2000). This species is widely cultivated worldwide for oilseed production
118 and as food crops. In pollinator surveys carried out in New Zealand and Java (Indonesia), *B. rapa*
119 showed a generalist pollination system with a wide variety of pollinators belonging to the orders
120 Hymenoptera, Diptera, Lepidoptera and Coleoptera (Atmowidi *et al.* 2007; Rader *et al.* 2009).
121 *Brassica rapa* is commonly attacked by *Pieris brassicae* caterpillars, a native specialist herbivore,
122 whose caterpillars are specialized in feeding on the Brassicaceae family and have adapted to
123 overcome the glucosinolate defense system, typical of this plant family (Smallegange *et al.* 2007).
124 Interestingly, butterflies from the genus *Pieris* also act as pollinator (e.g. Rader *et al.* 2009). In
125 natural populations, *Brassica rapa* is also attacked by generalist herbivores such as the native
126 herbivore *Mamestra brassicae*, or the alien herbivore *Spodoptera littoralis*, a polyphagous moth,
127 native to Egypt. *S. littoralis* is considered a major pest for a wide variety of host plants (Amin &
128 Gergis 2006) and since the 1950's it has spread around the Mediterranean basin causing serious
129 damage to crops (CABI Invasive Species Compendium: <https://www.cabi.org/isc/datasheet/51070>).
130 This herbivore was also reported to be able to feed on *B. rapa* leaves (Schiestl *et al.* 2014;
131 Chabaane *et al.* 2015).

132

133 Study area and biological material

134 All experiments were conducted in a meadow of the Naples University Campus of Monte
135 Sant'Angelo (hereafter referred to as MSA). This meadow is at the edge of a densely inhabited
136 area and can be considered a disturbed habitat. *Brassica rapa* seeds were collected in a semi-
137 natural population from Roccamonfina (population size over 1000 individuals, Southern Italy).
138 Seed germination and plant growth were carried out in pots under standardized light (L16:D8), soil
139 and watering conditions in the greenhouse of the Biology Department at MSA.
140 Eggs from *P. brassicae*, *M. brassicae* and *S. littoralis* were obtained in different ways. For *P.*

141 *brassicae*, female butterflies were caught with insect nets in the MSA Campus, placed in net cages
142 and fed with a sugar solution. *B. rapa* plant was inserted in the cage to serve as an egg deposition
143 site. For *M. brassicae* and *S. littoralis*, lines of the individuals used in our experiments, instead,
144 originated from populations reared at the Swedish University of Agricultural Science (Alnarp,
145 Sweden) and refreshed with new wild-collected individuals approximately every 6 months. *M.*
146 *brassicae* and *S. littoralis* eggs were placed in a climate chamber at 22 °C, 60% relative humidity
147 and L18:D6 photoperiod in the Department of Biology at MSA. After hatching, first instars were
148 reared on an artificial diet (Elzinga *et al.* 2003) and the second instars (after 3–4 days) were fed
149 with young leaves of *B. rapa*.

150

151 Herbivory application

152 *Brassica rapa* individuals were exposed to different treatments: infested (i) by *Pieris brassicae*, (ii)
153 by *Mamestra brassicae*, (iii) by *Spodoptera littoralis* or (iv) not infested, as control treatment
154 (hereafter referred to as P-infested, M-infested, S-infested and control plants, respectively). To
155 apply herbivory on *B. rapa* individuals, we used the approach described in Litto *et al.* (2015).
156 Briefly, feeding was limited to a single leaf by containing caterpillars in clip cages made of Petri
157 dishes at the beginning of the bolting stage. Feeding was monitored regularly, and the caterpillars
158 were replaced, if necessary. In the control group, empty clip cages were applied as the control
159 treatment. P-infested, M-infested and S-infested plants were infested with two to six caterpillars
160 (depending on larval instar), five days before flowering (i.e. when the flowering shoot was c. 10 cm
161 long). Damage imposed by herbivorous feeding was estimated from each plant of the three
162 herbivory treatments in 2015, taking a picture of damaged leaves with graph paper as reference.
163 Before photographing, leaves were pressed with a transparent slide to avoid three-dimensionality.
164 Leaf damage was then measured using ImageJ software.

165

166 Experimental design

167 To estimate the effect of native (*P. brassicae* and *M. brassicae*) vs alien (*S. littoralis*) herbivores on
168 pollen movement mediated by local pollinator communities, we conducted two different

169 experiments. The first experiment (hereafter referred to as pollen staining plots) was conducted in
170 2015. We established seven experimental plots with 20 plants, i.e. five individuals for each
171 herbivore treatment (P-infested, M-infested, and S-infested) and five control plants. For each
172 treatment, pollen from one plant was stained using a fluorescent dye. We used Orange, Blue,
173 Green and Pink dyes, alternating colours for the different treatments in the different plots to avoid a
174 “colour effect” (Van Rossum *et al.* 2011). In each plot, the position of plants was randomized. To
175 avoid pollination, plants used in this experiment were kept under a net in the greenhouse until
176 being used for experimental plots. Plots were exposed separately to local pollinators in the
177 meadow at MSA for two to six hours each (see Table S1 for details). We spaced *B. rapa* plants
178 within the plots of 0.5 m to avoid pollen being transferred through physical contact between
179 neighbouring plants. After exposure, plants were moved into a dark room and observed under UV-
180 light. We noted the plants that were visited by insects carrying stained pollen and the maximum
181 and average distance of stained pollen transferred among plants within each plot. Flight distance
182 within a plot can range from 0.5 (i.e. minimum distance between two plants) to 1.92 m (i.e.
183 maximum distance between two plants).

184 To disentangle the role and the foraging preferences of honey bees and other pollinators facing
185 different treatments, we conducted a second experiment (hereafter referred to as pollinator choice
186 plots) in spring 2017 where we directly observed the behaviour of each pollinator. Since *M.*
187 *brassicae* imposed significantly more damage to plants (Fig. S1), impeding a comparison with the
188 effects of the two other herbivores, we focussed on the native specialist vs invasive herbivores (i.e.
189 P-infested vs S-infested and control plants treatments). Four plots were built with 15 plants
190 randomly placed, i.e. five plants per treatment. For each plot, 5 days after the beginning of the
191 herbivory application, individuals were transferred from the greenhouse to the meadow. This
192 experiment was realized on different dates over a period of approximately three weeks to cover the
193 seasonal pollinator *spectrum* (May 19, May 29, June 1, June 8). Before exposure to pollinators,
194 phenotypic measures were taken from each plant. We measured the following floral traits: petal
195 length, petal width, sepal length, and sepal width. We also estimated plant maximum height and
196 the total number of open flowers, allowing to make a comparison between P-infested and S-

infested plants. In this experiment, it was not possible to standardize the plant age of control plants and infested individuals. Indeed, whilst infested plants were in the same phenological stage (due to herbivore application 5 days before the beginning of the flowering), control plants were chosen among the plants flowering in the greenhouse. Plots were then exposed to pollinators during three hours in the meadow at MSA and direct observations were realized for each of the four plots. As previously, plants were placed at a distance of 0.5 m to avoid physical contact. All the pollinators foraging the flowers were individually observed (determination, count, sequence of all the visited plants) walking in the plot for three hours. Each insect that could not be directly recognized was collected and stored in ethanol for subsequent identification. We tallied the number of visits and calculated the visitation rate for each treatment for both insect groups, i.e. honey bees and other wild pollinators, as the ratio between number of visits and total number of visits.

208

209 Data analysis

To understand the impact of different herbivores on *B. rapa*, we estimated leaf damage from each herbivore treatment (P-infested, M-infested and S-infested) in 2015 and tested the difference between them using the Kruskal-Wallis test and a Dunn Kruskal-Wallis multiple comparison with p-value adjust with Bonferroni method (R package FSA) for pairwise comparisons.

For the pollen staining plots, we calculated the probability of pollen from a given treatment to be exported onto plants exposed to the same or different treatments. For instance, the probability of pollen from a P-infested plant being exported onto another P-infested plant was calculated for each plot as the number of P-infested plants receiving P-infested pollen relative to the total number of plants receiving P-infested pollen. To test whether the herbivory treatment impacts on pollen export, we then compared the probability of pollen export and the average distance of stained pollen movement within each plot for each treatment using the Kruskal-Wallis test and a Dunn Kruskal-Wallis multiple comparison with p-value adjust with Bonferroni method (R package FSA) for pairwise comparisons.

For the pollinator choice plots, we observed the impact of the herbivory on plant phenotypic traits by comparing phenotypic traits among treatments with a Kruskal-Wallis test. To test the effect of

225 different traits on pollinator visitation rates, we correlated phenotypic traits that showed significant
226 differences between treatments with the number of visits using the Spearman's method. To
227 understand if herbivore treatments influence honey bees or other wild pollinator visitation rates, we
228 compared the number of visits to each treatment between honey bees and other wild pollinators
229 using the Wilcoxon test and performed a Chi Square test. Finally, we tested whether the herbivore
230 treatment influences the choice of honey bees or other wild pollinators to return preferentially to a
231 plant with the same treatment or to another plant, using the Wilcoxon test.

232 All the analyses were performed using R environment (Version 1.1.423 – © 2009-2018 Rstudio,
233 Inc.).

234

235 **RESULTS**

236 Herbivory damage

237 Whilst the leaf damage imposed by *Mamestra brassicae* was higher than that caused by other
238 herbivores (Kruskal-Wallis, Chi Square 13.53; DF = 2; $P = 0.001$), we found no significant
239 differences in herbivory damage imposed by the native *Pieris brassicae* and the invasive
240 *Spodoptera littoralis* ($Z=1.37$, $P_{adj}=0.51$; Fig. S1).

241

242 Pollen staining plots

243 No significant differences in the probability of pollen export were observed among control plants,
244 P-infested, M-infested and S-infested plants (Chi Square 14.77, Df=15, $P=0.45$; Fig. 1a). For a
245 given treatment, no significant differences were observed between the probability of revisiting the
246 same plant treatment or to visit a different one, despite a non-significant trend for P-infested plants
247 that are less likely to export pollen on other P-infested plants (control plants; Chi square=1.44,
248 Df=3, $P=0.70$, P-infested plants; Chi square=6.30, Df=3, $P = 0.1$, M-infested plants; Chi
249 square=4.72, Df=3, $P=0.19$, and S-infested plants; Chi square=1.70, Df=3, $P=0.64$; Fig. 1b).
250 The average flight distance for all treatments was 1.16m (min=0.5, median=1.16, max=1.92) and
251 was similar for each treatment (control plants mean=1.20m; P-infested mean=1.07m; M-infested
252 mean=1.13m; S-infested mean=1.24m). The different treatments showed no significant differences
253 in terms of pollen dispersal range (Chi Square 4.34; Df = 3; $P = 0.23$; Fig. 1c).

254

255 Pollinator choice plots

256 In the four pollinator-choice plots we individually observed 104 pollinators of *B. rapa* belonging to
257 21 taxa. The most abundant pollinators were honey bees (*Apis mellifera*, $n = 33$, i.e. 31.7% of the
258 total number of insect pollinators; Fig. S2). Excluding honey bees, and only considering other wild
259 pollinators, Hymenoptera were the main pollinators ($n = 47$ out of 71, 66.2%), but the proportion of
260 Diptera ($n = 11$ out of 71, 15.5%), Coleoptera ($n = 7$ out of 71, 9.9%) and Lepidoptera ($n = 6$ out of
261 71, 8.5%) was not negligible (Fig. S2). Honey bees visited on average 3.21 plants (min=1,
262 median=2, max=21) whilst other Hymenoptera 3.20 plants (min=1, median=1, max=39), Diptera

2.72 plants (min=1, median=1, max=7), Coleoptera 1.14 plants (min=1, median=1, max=2),
Lepidoptera 2.83 plants (min=1, median=2, max=8). For further comparisons, we grouped wild
Hymenoptera, Diptera, Coleoptera and Lepidoptera as opposite to the honey bees (*Apis mellifera*).
Floral traits showed no significant differences among treatments (i.e. control plant, P-infested and
S-infested; Petal width: Chi square = 0.24, DF = 2, $P = 0.887$; Petal length: Chi square = 0.76, DF
= 2, $P = 0.683$; Sepal width: Chi square = 0.37, DF = 2, $P = 0.831$; Sepal length: Chi Square =
3.71, DF = 2, $P = 0.156$; Fig. S3). S-infested plants were higher than P-infested plants but not
significantly (mean height of P-infested plants = 68.1 mm; mean height of S-infested plants = 58.2
mm; Fig. S4) and the two herbivore treatments showed no differences in terms of number of open
flowers (mean number of open flowers of P-infested plant = 61.2; mean number of open flowers of
S-infested plant = 63.9; Fig. S4). Comparison tests showed significant differences in the height of
control plants with respect to S-infested plants (control vs S-infested; $Z = 4.04$, $P_{\text{adj}} = 0.0002$, Fig.
S4), and in the number of open flowers between control and both herbivory treatments (control vs
P-infested plant $Z = 3.41$, $P_{\text{adj}} = 0.0019$, control vs S-infested plant $Z = 3.00$, $P_{\text{adj}} = 0.0082$, Fig. S4).
The number of open flowers is significantly correlated with the number of pollinator visits. This
correlation was negative for honey bees ($\rho = -0.29$, $P = 0.0317$) and positive for other wild pollinators
($\rho = 0.45$, $P = 0.0006$). Within different treatments, we found significant correlations only in control
plants for other wild pollinators (honey bees: $\rho = -0.36$, $P = 0.1146$, other wild pollinators: $\rho = 0.64$,
 $P = 0.002$, Fig. 2a and 2b). Correlations were instead non-significant in P-infested and S-infested
treatments for both honey bees and other wild pollinators (Fig. 2a and 2b). All the plant treatments
attracted the same pollinator set (including Hymenoptera, Diptera, Coleoptera and Lepidoptera).
Honey bees showed no preference for different treatments (35 visits on control plants, 34 on P-
infested plants, 35 on S-infested plants; Chi square=0.02, Df=2, $P = 0.9903$; Fig. 3a), whilst other
wild pollinators tend to visit preferentially, but not significantly, control plants (80 control, 65 P-
infested and 56 S-infested plants; Chi square=4.39, Df=2, $P = 0.112$; Fig. 3a). Honey bees and
other wild pollinators also showed non-significant differences in terms of choice of the first plant in
the plot (honey bees; Chi square=1.64, Df=2, $P = 0.44$, other wild pollinators; Chi square=2.82,
Df=2, $P = 0.24$, Fig. 2b). Overall, in terms of visits (first one or during all the experiment), we found

that other wild pollinators contributed more than honey bees, performing significantly more visits to control and P-infested treatments (all treatments: $W=842.5$, $P= 0.00004$, control: $W=98$, $P= 0.00513$, P-infested: $W=74.5$, $P= 0.01405$; S-infested: $W=111.5$, $P= 0.1042$, Fig. 2c). At pollinator community level, after visiting a control plant, there was an equal probability of visiting any treatment ($W=4983$, $P= 0.1373$). When separating honey bees from other wild pollinators, after visiting a control plant, the honey bees significantly prefer visiting a different plant treatment to revisiting another control plant ($W=430.5$, $P=0.035$, Fig. 3c). This trend was not observed for other wild pollinators ($W=2471$, $P=0.7551$, Fig. 3c). At pollinator community level, after visiting a P-infested plants there was a lower probability of visiting another P-infested plant than another treatment ($W = 4809$, $P = 0.02195$); however, this pattern was not significant when analysing honey bees and other wild pollinators independently (honey bees; $W=459$, $P=0.1155$, other wild pollinators; $W=2296$, $P=0.09588$, Fig. 3c). At pollinator community level, after visiting a S-infested plant there was a lower probability of visiting another S-infested plant than another treatment ($W = 4419.5$, $P = 0.00062$); this pattern was significant for honey bees ($W = 358.5$, $P = 0.00181$) but not for other wild pollinators ($W = 2238.5$, $P = 0.0574$; Fig. 3c).

307

308 DISCUSSION

Whilst pollinator attraction and herbivore repellence may be strongly interconnected due to common signals (or common metabolic pathways) used by plants to communicate with mutualistic and antagonistic insects, most studies investigated them separately (Desurmont *et al.* 2014). Here we tested the effect of both native and alien herbivores on the local pollinator community of the generalist species *Brassica rapa* and disentangled the role of honey bees *versus* other wild pollinators in plant pollen movements.

By staining the pollen of *B. rapa* plants infested by different herbivores (native specialized, native generalist or alien), we determined pollinator movement among treatments and observed the fine-scale pollen dispersal range. We found that herbivore identity does not alter intra and inter-treatment pollen movements nor pollen dispersion distance. Pollen collected from a plant exposed

319 to herbivore treatment had similar chances of reaching a plant infested by *Pieris brassicae*,
320 *Mamestra brassicae*, *Spodoptera littoralis* or a control plant. The only exception was observed for
321 P-infested plants that appeared to export less pollen to other P-infested plants (Fig. 1). This pattern
322 was marginally significant but likely suggests an avoidance behaviour of the pollinator community
323 of plants infested by herbivores as already reported in Kessler *et al.* (2011) and in Schiestl *et al.*
324 (2014). Control and treated plants showed no significant differences in terms of pollen dispersal
325 range (Fig. 1). However, as the maximum distance between plants within plot was less than two
326 meters, this finding is more indicative of fine-scale insect choices between different herbivore
327 treatments than of pollen transfer among distant plant patches.

328 In generalist species such as *B. rapa*, the overall pollination can be shaped by different
329 contributions from different pollinators. We found that even in a disturbed and fragmented habitat
330 as MSA, *B. rapa* attracts a noteworthy diversity of pollinators (Fig. S2). Overall, concordantly with
331 the previous findings (Atmowidi *et al.* 2007; Rader *et al.* 2009), honey bees were the most common
332 pollinators (31.7%). Among other wild pollinators, Hymenoptera accounted for the highest
333 contribution, even if Diptera, Coleoptera and Lepidoptera were also responsible for a significant
334 proportion of pollen export. All plant treatments attracted all the pollinator groups, though honey
335 bees and other wild pollinators showed different preferences in response to different plant
336 treatments (i.e. control plants, P-infested and S-infested plants). Indeed, other wild pollinators
337 visited comparatively more control plants, though not significantly, whilst honey bees showed no
338 preferences and visited any treatment with the same frequency (Fig. 3). An increased
339 attractiveness of control plants to pollinators was already observed in previous studies where
340 bumble bees forage preferentially uninfested *B. rapa* plants likely mediated by visual and
341 especially olfactory floral signals (Schiestl *et al.* 2014; Kellenberg *et al.* 2016). However, these
342 studies were conducted in an experimental setup including only one pollinator type (i.e. bumble
343 bees), despite the fact that *B. rapa* is a generalist species. Our results suggest that though wild
344 pollinators can perceive the signals produced by infested plants, preferring control individuals,
345 honey bees do not rely on the same signals when foraging. Altogether, the combined action of
346 honey bees and other wild pollinators is likely to mitigate the preferential behaviour of wild

347 pollinators, hence leading to slight differences in pollen export, in movement between infested and
348 control plants and probably in plant seed set, as also found in *Brassica nigra* by Rusman *et al.*
349 (2018).

350 Whilst it was possible to estimate the overall movement of pollen in the pollen staining plots, the
351 pollinator choice plots allowed the observation of the shifts of pollinators between plants by tracing
352 pollinator foraging behaviour after the first visited treatment. Even for this behaviour, we found
353 differences between honey bees and other wild pollinators with the former tending to avoid
354 revisiting the same treatment and the latter showing no avoidance behaviour (Fig. 3). This finding
355 suggests that the effects of herbivory on plant pollination in a generalist species as *B. rapa* can
356 depend on the composition of the pollinator community. By analysing the data at community level
357 (i.e. honey bees and other wild pollinators together) we found a lower probability of P-infested
358 plants to export pollen on other P-infested plants thus confirming the lower number of events of
359 pollen movement among P-infested plants observed in the pollen staining experiment.

360 Interestingly, this pattern is not significant when analysing honey bees and other wild pollinators,
361 independently suggesting that it can be due to an additive effect of non-significant trends.

362 Differently from what we observed in the pollen staining experiment, in the pollinator choice plots
363 we found significant avoidance behaviour after visiting a S-infested plant. This finding was due to
364 honey bees avoiding other S-infested plants and preferentially visiting control and P-infested
365 plants. This behaviour was likely masked, in the pollen staining experiment, by the cumulative
366 effect of different foraging behaviour of honey bees and other wild pollinators. Such detected
367 differences in foraging behaviour between honey bees and other wild pollinators have been widely
368 reported in literature (e.g. MacKenzie 1994) and may be due to different “optimal foraging”
369 strategies (*sensu* MacArthur & Pianka 1966; Charnov 1976). Furthermore, honey bees and other
370 wild pollinators may influence each other’s foraging behaviours due to competition for floral
371 resources (Shavit *et al.* 2009).

372 In the pollinator choice experiment, we found significant differences in floral display among
373 different treatments (either in terms of plant height and of flower number; Fig. S4). Changes of
374 flower display can be due to a resource allocation strategy following the herbivore attack (Elmqvist

375 & Gardfjell 1988; Quesada *et al.* 1995; Mutikainen & Delph 1996) and, in principle, can affect
376 pollinator visiting behaviours (Schiestl *et al.* 2014; Cozzolino *et al.* 2015). In our experimental setup
377 we were unable to detect whether such differences were consequence of the herbivore attack or of
378 a non-standardized plant age of control plants (randomly chosen among available flowering control
379 plants). Nevertheless, to detect the effect of flower display on visiting behaviour we calculated the
380 correlation between number of visits and number of open flowers for honey bees and for other wild
381 pollinators, separately. We found a significant positive correlation between number of visits and
382 number of open flowers for other wild pollinators and a not significant negative correlation for
383 honey bees (Fig. 2). This finding suggests that other wild pollinators, differently from honey bees,
384 can select the plant to visit also depending on flower number. However, at individual level, other
385 wild pollinators do not show selective preference for higher flower number of the first visited plant
386 (Fig. 3, Fig. S4) and the positive correlation between number of visits and number of open flowers
387 for other wild pollinators was only significant for control plants (Fig. 2). This evidence suggests
388 that, in our experimental setting, number of open flowers is not likely to be an important factor in
389 shaping wild pollinator behaviour. This is also supported by previous experiments demonstrating a
390 predominant role of floral volatile compounds in affecting post-herbivory attractiveness of *B. rapa*
391 (Schiestl *et al.* 2014).

392 Even if, with the present experimental design, we were unable to identify the visual or olfactory
393 plant traits conditioning the different pollinator behaviours, taken together our results suggest that
394 the different proportion of honey bees and other wild pollinators can lead to different selective
395 pressures and call for future studies on *B. rapa* (or other generalist plant species) pollination by
396 manipulating relative proportions of honey bees and other wild pollinators. Furthermore, the three
397 herbivores used in our study have different ecological and behavioural characteristics beyond their
398 origin and history of coexistence with *B. rapa*. Thus, future studies, should preferentially also
399 employ pairs of phylogenetically-related native and invasive herbivores, to dissect whether the
400 effects induced by herbivores on pollination can be due to their origin or to any other ecological
401 difference.

402

403 **ACKNOWLEDGMENTS**

404 Authors thank Swedish University of Agricultural Science (Alnarp, Sweden) for providing *Mamestra*
405 *brassicae* and *Spodoptera littoralis* eggs; Mary Longrigg and Emily Scopece for language revision;
406 Maria Litto and Martina Campolo for help during fieldwork. Florian Schiestl, Karl Duffy and two
407 anonymous reviewers provided very helpful comments on an earlier version of the MS. This
408 research was carried out in the frame of Programme STAR, financially supported by UniNA.

409

410

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572 **Figure legends**

573

574 **Figure 1.** Pollen staining plots: probability of pollen export (a) inside each treatment (Control for
575 control plant, P-infested for plants infested by *Pieris brassicae*, M-infested for plants infested by
576 *Mamestra brassicae*, and S-infested for plants infested by *Spodoptera littoralis*), or (b) from a given
577 plant treatment to any other plant treatment. Each pollen movement combination is designated by
578 two letters: the first indicates the pollen source, the second the pollen receiver (C = control plants,
579 P = plants infested by *P. brassicae*, M = plants infested by *M. brassicae*; S = plants infested by *S.*
580 *littoralis*). (c) Pollen dispersal distance of stained-pollen from each treatment.

581

582 **Figure 2.** Correlation between the number of open flowers and the number of visits for (a) honey
583 bees and (b) other wild pollinators. (c) Number of visits of honey bees (black) and other wild
584 pollinators (grey) to plants exposed to different treatments. Non-significant values are indicated in
585 light grey and significant values in black. * $P < 0.05$, ** $P < 0.01$.

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587

588 **Figure 3.** Pollinator choices facing different herbivory treatments. (a) Total number of visits by
589 honey bees (left panel) and other wild pollinators (right panel) to the three different treatments
590 (Control plants; P-infested: plants infested by *Pieris brassicae*; S-infested: plants infested by
591 *Spodoptera littoralis*); (b) Number of honey bees (left panel) and other wild pollinators (right panel)
592 visiting the three different plant treatments as first choice. (c) Pollinators shifts among plants
593 exposed to different treatments for both categories of pollinators (honey bees on left panel and

594 other wild pollinators on right panel). Each pollen movement combination is designated by two
595 letters: the first indicates the pollen source, the second the pollen receiver (C = control plants, P =
596 plants infested by *P. brassicae*, M = plants infested by *M. brassicae*; S = plants infested by *S.*
597 *littoralis*).

598

599 **Supporting information**

600 **Table S1.** Date, time of exposure to pollinators and total number of dye movements for the seven
601 pollen staining plots.

602

603 **Figure S1.** Comparison of herbivore damage in plants infested by the wild generalist *Mamestra*
604 *brassicae*, the wild specialised *Pieris brassicae* and the invasive generalist *Spodoptera littoralis*.

605

606 **Figure S2.** Pollinators observed in the experimental plots and their relative abundance.

607

608 **Figure S3.** Comparison of floral traits (a) Petal width, (b) Petal length, (c) Sepal width (d) Sepal
609 length, among plants exposed to different treatments (Control plants, P-infested: plants infested by
610 *Pieris brassicae*, S-infested: plants infested by *Spodoptera littoralis*).

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612 **Figure S4.** Comparison of plant height (a) and number of flowers (b) for the different herbivore
613 treatments.

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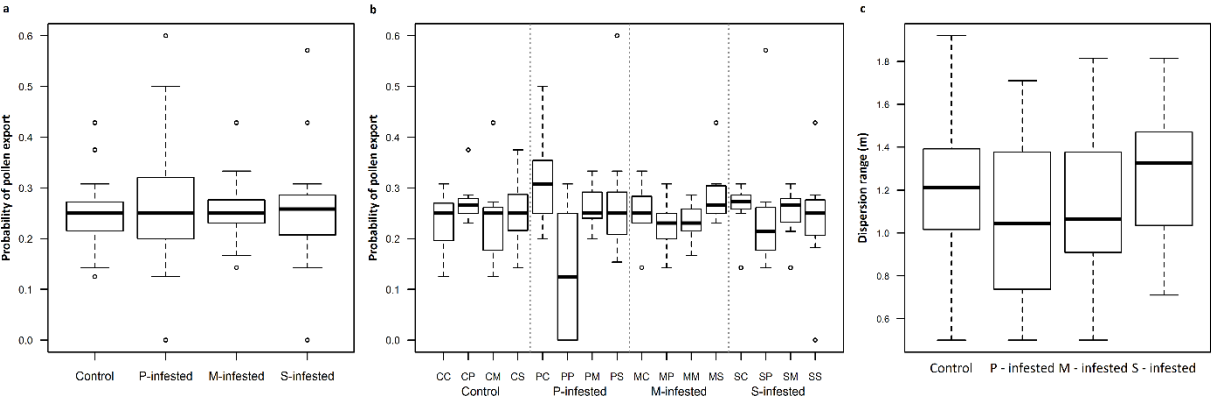
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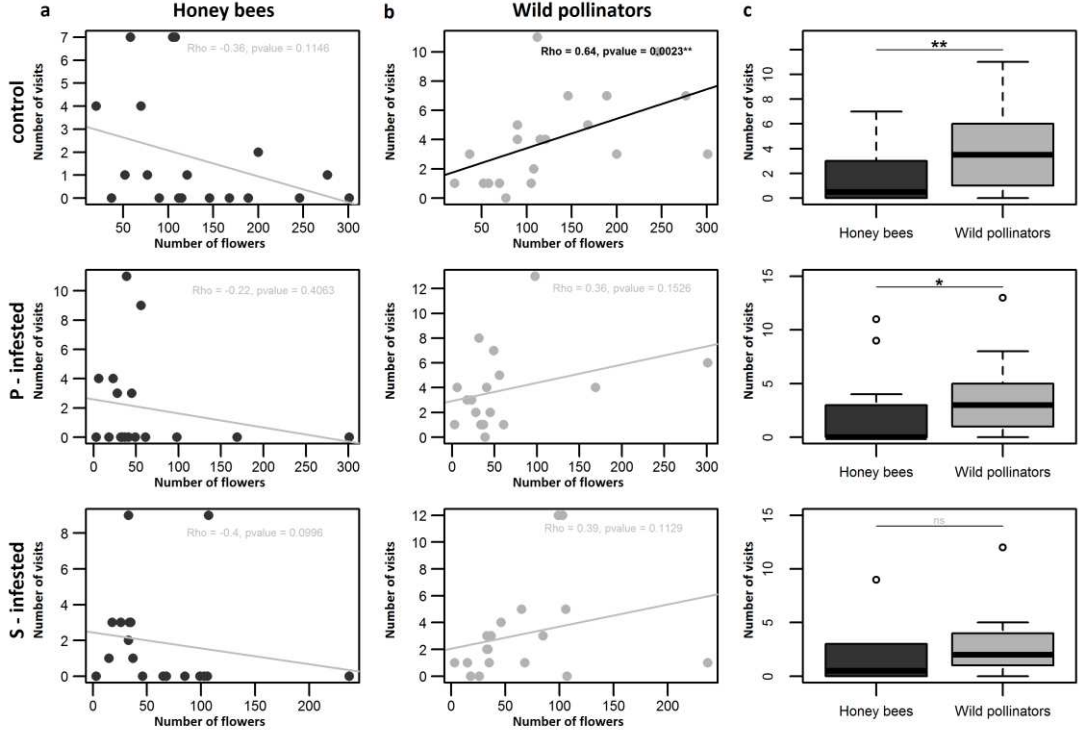
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630 **Figure 1**



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634 **Figure 2**



652 **Figure 3**
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